Opponent review of the thesis

M.Maděra:

Development of method for High-Throughput Enrichment of Glycoproteins and Glycopeptides Emploing Multiple Lectin Affinity Chromatography/Tandem Mass Spectrometry

This thesis summarized the development of a multimetodological analytical approach emploing microcolumn lectin affinity chromatography coupled to high-resolution separation and detection techniques:

- Silica-based lectin affinity chromatography and their incorporation into semiautomated, high-throughput system
- enrichment of glycopeptides and glycoproteins present in real samples
- in conjunction with immunoaffinity depletion, high-recovery RPLC fractionation and tandem mass spectrometry
- identification of some glycoproteins expressed at pg/mL concentration level

Aim of tis study was the development of multidimensional system based on microcolumn lectin affinity chromatography coupled on-line to high-resolution separation and detection techniques.

Literature review is well arranged, theoretical part is extensive, structuring of the work is in accordance with the custom practice. The methods selected are in accordance with the demands. Author got a lot of different methodics under control. Experimental results, the way of their fruition and interpretation are very valuable. Autoreview is very well arranged and has high informative value, meet the requirements of promulgation and contain all the appropriateness.

The work is extremely topical, the development of proteomics need new analytical methods at present. Coupling high-performance microcolumn affinity chromatography on-line to highresolution separation and detection techniques are important not only for the basic research, but for the matter for the possible utilization in diagnostics of serious civilizing diseases.

Thesis meet all the requirements of the public notice, candidate, no doubt, possesses the scientific methods, has profound theoretical knowledge and his work, as saied before, bring new scientific attainments.

My remarks:

- Reason of using silica gel as affinity carrier for HPLC (comparison with other HPLC supports)
- Nonspecific sorption at described conditions of chromatography
- Have you some information from test of this system for glycoproteomic distinction between normal and pathological samples?

I recommend the work for defense.

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